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DIEGO LEANDRO DE OLIVEIRA

ASPECTOS ECOLÓGICOS E COMPORTAMENTAIS DE MUSCOIDES (DIPTERA) EM AMBIENTES DO NORDESTE BRASILEIRO

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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco como requisito parcial para a obtenção do título de Doutor em Biologia Animal.

Área de concentração: Biologia Animal

Orientador: Prof. Dr. Simão Dias de Vasconcelos

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RESUMO

Ecologia e comportamento de moscas necrófagas são temas complexos para serem estudados de forma única. Sendo assim, esta tese foi projetada e desenvolvida em três capítulos de forma a abordar esses temas com base no direcionamento *situações estressantes*. No primeiro capítulo, foi observada a estratificação de voo de duas espécies de califorídeos na Caatinga, uma floresta sazonalmente seca de clima semiárido. Para observar o voo, baseado na captura dos insetos, o dia foi dividido em quatro tratamentos de três horas cada (das 05:30 às 17:30), além de um tratamento noturno das 17:30 às 05:30. Diferente do esperado, as espécies *Chrysomya albiceps* e *Cochliomyia macellaria* tiveram um padrão de voo semelhante, com menor abundância nas horas de temperatura mais amenas do dia, e maior abundância nas horas mais quentes e secas. Além disso, não houve registro de voo noturno para estas espécies. Os resultados contribuem para o conhecimento sobre a ecologia de moscas necrófagas, e potencialmente para o desenvolvimento da Entomologia Forense na Caatinga, uma vez que espécies competidoras chegam ao recurso ao mesmo tempo, independente do horário. O segundo capítulo trata de um tema ainda negligenciado na literatura, o comportamento de voo vertical de moscas necrófagas em estratos elevados. Utilizando como modelo experimental prédios não concluídos de Recife (Pernambuco), observou-se a ocorrência de dípteros necrófagos em armadilhas expostas no andar térreo (1,5 m) até o 27° (85 m). Como esperado, houve uma maior abundância de moscas próximo ao solo (quase 80%), sendo Calliphoridae a família mais abundante (53%). Quanto à distribuição vertical, Calliphoridae e Muscidae foram registradas do térreo ao 15° andar (48 m), Sarcophagidae até o 21° (67 m) e Phoridae até o 27° (85 m). Este foi o primeiro experimento quali-quantitativo e replicado a estudar a estratificação vertical de voo de espécies sinantrópicas no continente americano, com potencial aplicabilidade na entomologia médica, veterinária e forense para ambientes urbanos. O terceiro e último capítulo relata a investigação do processo de colonização de um cadáver humano parcialmente suspenso, e a dinâmica envolvendo a presença de larvas diretamente sobre o cadváver e no solo circunvizinho. As larvas foram coletadas de acordo com tratamentos espaciais artificiais que incluem cabeça e o pescoço, os membros superiores, o solo imediatamente abaixo do corpo e o solo afastado do corpo em até 1,5 m. Foram identificadas seis espécies de moscas colonizando o cadáver, com destaque para *Chrysomya albiceps* (97% do total) e *Fannia pusio*, sem registros quantitativos prévios em cadáveres. Este capítulo consolida uma colaboração da Universidade com a Polícia Civil de Pernambuco e reforça o enorme potencial para o desenvolvimento da Entomologia Forense nesta região. Espera-se com esta tese responder a perguntas importantes acerca do comportamento e ecologia de moscas necrófagas sob situações estressantes, bem como estimular o desenvolvimento de pesquisas complementares, que deem continuidade a uma perspectiva interdisciplinar da Entomologia Aplicada.

Palavras-chave: Moscas necrófagas. Entomologia Forense. Caatinga. Voo. Intervalo pós-morte.

ABSTRACT

Ecology and behavior of necrophagous flies are complex subjectsto be studied in a single approach. Therefore, this thesis was designed and developed in three chapters in order to address these topics under the scope of *stressful situations*. In the first chapter, the flight stratification of two species of califorids was observed in the Caatinga, a seasonally dry forest of semiarid climate. To observe the flight, based on the capture of the insects, the day was divided into four treatments of three hours each (from 05:30 to 17:30), in addition to a nocturnal treatment from 17:30 to 05:30. Different from the expected, the species *Chrysomya albiceps* and *Cochliomyia macellaria* had a similar patterns of flight, with a lower abundance in the cooler hours of the day, and peak of the capture in the hotter and drier hours. In addition, there was no nocturnal flight record for both species. The results contribute to knowledge about the ecology of necrophagous flies, and potentially to the development of Forensic Entomology in the Caatinga, since competing species arrive at the resource at the same moment, regardless of the time. The second chapter addresses a subject still neglected in the literature, the vertical flight behavior of necrophagous flies in great heights. Using as experimental models unfinished buildings in Recife (Pernambuco), we observed the occurrence of necrophagous dipterans in traps exposed from the ground floor (1.5 m) to the 27th (85 m). As expected, there was a greater abundance of flies near the ground (almost 80%), with Calliphoridae as the most abundant family (53%). As for the vertical distribution, Calliphoridae and Muscidae were recorded from the ground floor to the 15th floor (48 m), Sarcophagidae to 21st (67 m) and Phoridae to 27th (85 m). This was the first replicated qualitative experiment to study the vertical stratification of flight of synantropic species in the American continent, with potential applicability in medical, veterinary and forensic entomology for urban environments. The third and final chapter reports the investigation of the process of colonization of a partially hanging human cadaver, and the dynamics involving the presence of larvae directly on the body and on the ground around it. The larvae were collected according to artificial spatial treatments including head and neck, upper limbs, soil immediately below the body and soil away from the body up to 1.5 m. Six species of flies were identified, including *Chrysomya albiceps* (97% of total) and *Fannia pusio*, never quantitatively recorded in human cadavers. This chapter consolidates a collaboration of the University with the Civil Police of Pernambuco and reinforces the vast potential for the development of Forensic Entomology in this region. It is expected that this thesis will answer important questions about the behavior and ecology of necrophagous flies under stressful situations, as well as stimulate the development of complementary research, which shall continue an interdisciplinary perspective of Applied Entomology.

Keywords: Necrophagous flies. Forensic Entomology. Caatinga. Flight. *Post-mortem* interval.

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INTRODUÇÃO

A família Calliphoridae compreende cerca de 1.500 espécies de moscas necrófagas conhecidas como varejeiras, que estão distribuídas em praticamente todos os continentes. São extremamente importantes na decomposição de matéria orgânica animal, sendo o principal grupo da ordem Diptera associado à decomposição de cadáveres humanos (CARVALHO et al., 2012). Por isso têm sido utilizadas como evidências em eventos envolvendo suspeitas de crimes como homicídios, sequestros e negligência, para obtenção de informações úteis à investigação criminal, aspectos fundamentais utilizados pela Entomologia Forense (EF) (CATTS e GOFF, 1992).

Estes insetos podem ser usados para a estimativa do Intervalo Pós-Morte (IPM), como evidência ou prova física de traslado *post-mortem* de cadáveres, casos de crimes ambientais e como indicadores de conservação ambiental (BYRD e CASTNER, 2010). As moscas desta família merecem destaque graças à sua alta abundância e riqueza, associadas à uma grande plasticidade alimentar, sendo atraídas por uma grande gama de iscas em decomposição. A composição da fauna tende a variar muito entre os ambientes amostrados, embora deva-se destacar o impacto das espécies do gênero *Chrysomya*, que invadiram o continente americano na década de 1970, e cujos espécimes têm dominado levantamentos faunísticos em vários ambientes do continente (GUIMARÃES et al., 1978).

A grande maioria dos estudos com moscas necrófagas realizados no Brasil são voltados para levantamentos faunísticos. Estes trabalhos, embora necessários, ignoram aspectos comportamentais destas espécies, traduzindo a necessidade de pesquisas que investiguem situações experimentais e/ou controladas. O repertório comportamental de moscas necrófagas é normalmente estudado em laboratório, o que permite uma grande replicabilidade e confiabilidade aos resultados, mas que ignora a resposta dos indivíduos a diversos fatores ambientais (abióticos), além da interação com outras espécies de insetos.

Um dos aspectos do comportamento de moscas necrófagas ainda pouco compreendido é a atividade de voo ao longo do dia e da noite. Na prática, entender como ocorrem as flutuações populacionais das moscas ao longo do dia pode ser útil para a investigação acerca do IPM, principalmente a capacidade de voo noturno, uma vez que a maioria dos crimes ocorre durante a noite. Este é um tema bastante controverso na literatura, uma vez que vários autores observaram de voo noturno (SINGH e BHARTI, 2001; WOOLDRIDGE, et al., 2007, SOARES e VASCONCELOS, 2016), enquanto outros não o registraram mesmo sob iluminação artificial (STAMPER, et al., 2009). Essa contradição sugere que características ambientais possam modular o voo, sendo necessário para uma aplicação mais plena pela EF, o mapeamento nos mais diversos ambientes, principalmente aqueles mais negligenciados por estudos comportamentais, como a Caatinga.

Além do período de atividade, a interação de moscas necrófagas com ambientes urbanos é, ainda, pouco compreendida. O processo de verticalização que têm ocorrido em grandes regiões metropolitanas como a de Recife (RMR), em Pernambuco, têm modificado drasticamente a paisagem natural, criando a oferta de substratos dispostos a diferentes alturas. O processo de detecção, localização, acesso e colonização de substratos localizados acima do solo é praticamente desconhecida. A recente colonização de um cadáver dentro de um apartamento na RMR (VASCONCELOS et al., 2014), tem dado ainda mais importância à necessidade de trabalhos que venham a explorar, experimentalmente, aspectos relacionados à estas questões, de grande potencial de aplicabilidade na EF.

A ausência do contato de substratos em decomposição com o solo, ainda que parcialmente (ex: carcaças penduradas, cadáveres enforcados) tem repercuções importantes na sua decomposição. Ao morrer, carcaças animais e cadáveres humanos passam a se decompor, atraindo as moscas, graças à atividade de microorganismos decompositores, inicialmente por aqueles já presentes no corpo, mas com o acréscimo imediato dos presentes no solo (AMENDT, et al., 2010). A suspenção de substratos, ainda que parcial, é uma situação estressante para insetos associados à decomposição, uma vez que muitos, como as larvas das moscas, não voam, e têm, portanto, dificuldade de acessibilidade.

Associar a ecologia ao comportamento de espécies de moscas necrófagas demanda a execução de experimentos de campo que objetivam entender uma determinada parcela do repertório de comportamental das espécies, ou até mesmo uma resposta a determinadas situações. Optamos aqui por avaliar a resposta de califorídeos a situações estressantes, sejam elas artificiais ou naturais. Há uma carência na literatura de estudos que avaliem a resposta de moscas necrófagas em situações de stress, seja ele térmico, de umidade, de altura, ou de acessibilidade ao substrato.

A Caatinga é um laboratório a céu aberto para a execução de estudos de campo sobre comportamento animal em ambientes estressantes. A baixa quantidade de substratos animais em decomposição mantém as populações de moscas necrófagas em baixa abundância, quando comparada com ambientes de florestas Atlântica ou Amazônica, mas em compensação, oferece baixa competição à oferta de substrato proporcionada pelos estudos. Com isso, espera-se amostrar mais facilmente grande parte da riqueza presente, ajudando a entender aspectos comportamentais de uma maior gama de espécies. Além disso, a Caatinga é o bioma brasileiro mais negligenciado por estudos científicos, representando menos de 1% das pesquisas no Brasil entre 1945 e 2008 (SANTOS et al., 2011), aumentando o potencial ineditismo dos resultados aqui obtidos.

A atratividade de um substrato a moscas necrófagas acontece devido à detecção, pelas moscas, dos Compostos Orgânicos Voláteis (COV) liberados pelo processo de decomposição (BYRD e CASTNER, 2010). Esta pista olfativa é forte o suficiente para moscas localizarem substratos a uma grande distância, ou até mesmo aqueles dispostos em ambientes fechados, como o interior de residências, por exemplo. Em ambientes naturais, moscas necrófagas encontram substratos dispostos à altura do solo, uma vez que animais mortos, mesmo em árvores, tendem a cair, mas há um turvo conhecimento acerca de sua capacidade de deslocamento vertical ao detectar prováveis substratos.

O contato com o solo é um fator fortemente influenciador do processo de decomposição. A ausência, ainda que parcial do contato com o solo permite uma menor interação com microorganismos decompositores, retardando a deterioração da matéria. Em caso de substratos pendurados, por exemplo, o processo de colonização pelas moscas é dificultado, uma vez que moscas adultas podem alcançar facilmente o substrato, mas os imaturos podem cair, se afastando da sua fonte nutricional. Este afastamento “forçado” influencia no seu desenvolvimento, consequentemente influenciando a sua utilização para o cálculo do intervalo pós-morte

1.1 OBJETIVOS

1.1.1 Objetivo geral

Avaliar a resposta de dípteros necrófagos da família Calliphoridae a situações estressantes, em ambientes representativos da região Nordeste do Brasil, integrando aspectos ecológicos (ex., riqueza, abundância, competição entre espécies nativas e invasoras), comportamentais (ex. horário de voo, altura de voo), fisiológicos (ex. colonização de substratos de difícil acesso), e forenses (ex., indicação de local de morte, status conservacionista de um ambiente).

1.1.2 Objetivos específicos

Avaliar a ocorrência de voo de moscas necrófagas (Calliphoridae) em diferentes alturas em diversas localidades de um centro urbano da região Nordeste do Brasil, observando como os padrões populacionais vão sendo afetados por estes fatores. Objetivando-se ainda: a) verificar como a população de califorídeos é afetada pelos tratamentos de altura; b) Investigar se o padrão populacional é o mesmo entre os locais amostrados; c) Avaliar a presença e a frequência relativa de espécies invasoras; d) Verificar se a razão sexual é semelhante entre os tratamentos de altura e de localidade.

Uma vez que há um turvo conhecimento acerca da atividade de voo de moscas necrófagas nos mais diversos ambientes, nos propomos a investigar as diferenças na atividade de voo ao longo do dia, e verificar se há registro voo durante a noite em um fragmento de Caatinga, baseado nos seguintes parâmetros: a) Estratificar a atividade de voo de califorídeos durante o dia, considerando a riqueza, abundância e frequência relativa; b) Comparar os registros de voo nos diversos horários, considerando fatores abióticos como temperatura e umidade relativa; c) Inferir sobre diferenças no comportamento de voo entre espécies nativas e invasoras; d) Observar se há registro de voo de algum espécime de Calliphoridae durante a noite. Este objetivo deu origem ao artigo científico reproduzido no apêndice A.

Analisar o processo de colonização de um cadáver humano vítima de suicídio por enforcamento, parcialmente pendurado na vegetação de um fragmento urbano de mata atlântica, registrando diferenças no desenvolvimento dos imaturos coletados de diversas partes do corpo e dos arredores do cadáver, considerando: a) analisar a diversidade de califorídeos capazes de colonizar o cadáver em um fragmento urbano de floresta Atlântica, atentando para a presença de espécies nativas e invasoras; b) Comparar a abundância e o tempo de desenvolvimento dos imaturos coletados no corpo em relação aos coletados no solo, próximos ao cadáver; c) Inferir acerca do Intervalo Pós-morte a partir do tempo de desenvolvimento dos imaturos. Este objetivo deu origem a um artigo científico já publicado, reproduzido no apêndice B.

2 REFERENCIAL TEÓRICO

Flies of the families Calliphoridae, Muscidae, Sarcophagidae and Phoridae (Diptera) play a key part in the cycling of organic matter, speeding up carrion decomposition (Byrd and Castner 2009). The ubiquitous presence of necrophilous flies in urban environments, however, heightens their role as physical vectors of a plethora of pathogenic bacteria, fungi, viruses, among other organisms (Nazni et al. 2005; Förster et al. 2007; Watson et al. 2007; Oghale et al. 2013). Some species, including the calliphorid *Cochliomyia hominivorax* (Coquerel, 1858), are capable of laying eggs on living animal tissues, which can cause myiasis in humans, pets and cattle (Nascimento et al. 2005; Byrd and Castner 2009).

The remarkable efficiency of necrophagous flies in locating and colonizing ephemeral resources results from behavioral traits involved in two major responses – the ability to detect chemical cues released from the resource and the ability to disperse towards it (Wall and Fisher 2001; Dekeirsschieter et al. 2009). Necrophilous dipterans meet these requirements and are among the first organisms to detect and reach carrion, colonizing it a few minutes after death (Vasconcelos et al. 2013). Blow flies, house flies, flesh flies and scuttle flies can find substrates that are concealed under different accessibility obstacles (Bhadra et al. 2014; Charabidze et al. 2015), and even buried (Simmons et al. 2010).

Most knowledge regarding accessibility of substrates by flies has been based on experiments settings under controlled conditions in the laboratory or, when in the field, at the ground level. For instance, it has been long known that flies can disperse for over 20 km in (horizontal) distances in order to find a suitable substrate (Bishopp and Laake 1921), but to this date there is no quantitative study about its vertical dislocation. When available, data on this subject refer to experiments performed at low heights, from a few centimeters to less than ten meters (Vogt et al. 1995; Bilaniuk and Beresford 2010; Roque et al. 2013).

On natural environments, suitable substrates decrease with height because carrion tends to lay on the ground. Considering that carrion insects also colonize alternative resources (e.g., rotting vegetable matter, feces) the spatial distribution may differ radically in urban environments, where man-produced resources can be available indoors – including in high-rise buildings. So far, most of the literature comparing indoor x outdoor flies' communities is also based in field trials set at the ground level (Reibe and Madea 2010; Martín-Vega et al. 2017).

Recently, cases in which flies were found colonizing human cadavers inside buildings started to draw attention to this scenario, as the flies were able to reach different heights in order to access the corpses (Abdullah et al. 2012; Vasconcelos et al. 2014; Syamsa et al. 2015). These first records enhanced the importance of designing replicated experimenting on vertical dislocation of flies beyond anecdotal registers (Abdullah et al. 2016; Heo et al. 2017).

The importance of understanding flies' upright dislocation is also emphasized by the fact that urban centers all over the world have gone through an accelerated landscape transformation over the past decades (García-Ayllón 2016), which culminated in a verticalization process, with the construction of high-rise and skyscrapers buildings, transforming the urban skyline. In Brazil, with over 210 million people, the human presence is irregularly distributed, with 76% of the population concentrated in highly urbanized areas (IBGE 2017), characterized by the presence of different kinds of buildings.

This study aimed to investigate the vertical distribution of necrophilous flies of the families Calliphoridae, Muscidae, Sarcophagidae and Phoridae, in an urban environment, using as experimental models uninhabited buildings. Specifically, we aimed to i) detect whether specimens from all families exhibit similarities in the ability to reach upper strata, and ii) compare the vertical distribution of blow fly species, under a medico-legal perspective. Our hypothesis was that the diversity and abundance of necrophilous flies tend to decrease in an inverse proportion to verticalization.

3 METODOLOGIA

3.1 AREA OF STUDY AND DESCRIPTION OF THE BUILDINGS

The experiment was performed in 2017 in Recife (8°03'47"S; 34°52'16"W), capital of the state of Pernambuco, in Northeast Brazil, which was selected as a model for this medico-legal entomology study due to three major factors. Firstly, it is the second most verticalized city in Brazil (Emporis 2019); secondly, it ranks amongst the most violent metropoles in the world (CCSPJP, 2017); and, lastly, as most of the high populated tropical cities, it suffers from high indices of vector-borne/insect-vectored diseases, due mostly to environmental, climactic and sanitation characteristics (Knudsen and Sloof 1991).

To measure the vertical stratification of the dipterans, we used as experimental models nine uninhabited buildings, distributed across four densely populated neighborhoods in the city (Figure 1). The outskirts of the buildings are predominantly composed by residencies, stores and roads subjected to intense traffic, with small stretches of mangrove or urban fragments of Atlantic rainforest.

We used buildings under construction, at similar degrees of conclusion, which were similar in design: “box-shaped” high-rise edifices, without structural setbacks. The buildings were fully accessible through open window frames and/or incomplete external walls and were not externally covered with safety nettings or other obstacles to the flies’ access. On the inside, all the doors and window frames were empty, and the walls (when present) were not fully erected, allowing free circulation of flies. Uninhabited buildings provided minimum interference resulting from human presence. Trash and other decomposing and possibly competitive organic matter, as well as deterrent substances such as household products and insecticides were absent. The same is valid to passive transportation of insects, as there were no elevators or other means of carrying insects upwards besides active flight.

3.2 EXPERIMENTAL DESIGN, COLLECTION AND IDENTIFICATION OF ADULT FLIES

To avoid misconceptions about “floors” and “stories”, we considered the ground floor as “0” level. Due to the logistical restraints associated with sampling all floors from the buildings, we decided to test one in every three floors from the 0 to the 27th level (when existing). This process was repeated for every edifice, that, depending on the height, had up to 10 individual tested floors.

In every sampled floor, we installed six suspended traps baited with 250 g of previously decomposed bovine spleen, according to methodology described elsewhere (Oliveira et al. 2016). The traps were hanged at 1.5 m from the floor of each level, reaching from 1.5 m (ground level) to ca. 85 m (27th level). The traps were preferentially hanged near external openings, protected from string wind, to facilitate the detection by the flies and at least one trap was positioned on each side of the floors.

To prevent competition and population depletion between across treatments or biases caused by structural changes inherent to the progression in the constructions, every floor was tested individually and only once. We performed the sampling in the weekends, to minimize interference from human activity. The exposure of the traps to the capture of dipterans occurred from a period of 60 ± 2 h, after which the captured flies were killed with Ethyl acetate vapor and stored in 70% Ethanol, until identification (Carvalho et al. 2002; Carvalho and Mello-Patiu 2008). Admission to the construction sites was authorized by the construction companies and all security and bioethics measures were strictly followed.

3.3 STATISTICAL ANALYSIS

To evidence conspicuous flight patterns, we divided the 10 tested floors into four major, non-overlapping strata, namely: Ground Section (ground floor), Low (3rd, 6th and 9th floors), Intermediate (12th, 15th and 18th floors) and High Section (21st, 24th and 27th floors). We described the adult dipteran assemblages captured according to the section, analyzing variables such as abundance and relative frequency of families and – in the case of Calliphoridae – species, according to each vertical treatment.

Differences in the abundance of insects between sections were tested through analysis of variance (ANOVA), after data transformation by either Square root (x) or Log (x + 1), until reaching the normality required by the test, which was confirmed by Kolmogorov-Smirnov tests. We also used Spearman's ranks correlations to verify and quantify the relationship between Diptera abundance and height.

The abundance data obtained with the collections was further transformed by Log (x + 1), followed by a for Bray-Curtis similarity analysis with the addition of a dummy value (= 1) to all samples, which was used to construct a non-metric multidimensional scaling (NMDS) graphic. The NMDS was implemented to show if there was formation of groups associated with the height treatments in the community structure. The results were further tested by the analysis of similarity (ANOSIM). All graphics and analyses were made using software BioEstat 5.0, Statistica 7, PRIMER 6 and Microsoft Office®, considering a significance level of 5%.

Figure 1 – Map of Recife showing the approximate location of the buildings used in the experiment (Bar = 1 km), and some examples of the exterior and interior of the buildings.

4 RESULTADOS

When the results of the 336 traps used in the experiment were combined, 10,701 adult flies belonging to the families Calliphoridae (52.9% of total specimens), Muscidae (41.2%), Sarcophagidae (3.2%) and Phoridae (2.7%) were collected. Regarding calliphorids, we identified seven species: *Chrysomya albiceps* (Wiedemann, 1819) (30.4%), *Ch. megacephala* (Fabricius, 1794) (68.3%), and, in much lower abundance, *Ch. putoria* (Wiedemann, 1818), *Cochliomyia hominivorax* (Cocquerel, 1858), *Co. macellaria* (Fabricius, 1755), *Lucilia eximia* (Wiedemann, 1819) and *L. sericata* (Meigen, 1826), that altogether represented 1.3% of the abundance of the family (Table 1).

The vertical distribution of insects' occurrence and abundance was more clearly evidenced when the individual heights sampled were merged into sections. The great majority of the specimens were captured on the Ground section (78.8%) and the abundance of sampled flies decreased until the High section, in which < 0.1% of the total was collected.

A decline in insects' abundance occurred as the height increased for specimens of the four families. For Calliphoridae, 84.6% of the flies were collected at the Ground section, with a steep decrease to the Low (14.2%) and Intermediate sections (1.2%), above which no individual was collected. Muscidae had 76.5% of the individuals collected at the Ground, decreasing to Low (21.2%) and Intermediate sections (2.2%), and no individual collected in the High section either. The vertical distribution of Calliphoridae ($F_{2,15} = 139.6$; $P < 0.01$) was similar to that of Muscidae ($F_{2,15} = 134.7$; $P < 0.01$); in both cases insects at the Ground section were significantly more abundant than in the Low and Intermediate sections ($P < 0.001$ for all) (Figure 2a and 2b, respectively).

The abundance of sarcophagids was as follows: at Ground Section (58.1%), Low (35.4%), Intermediate (5.6%) and 0.9% at the High Section (Table 1). For Sarcophagidae ($F_{3,20} = 52.0$; $P < 0.01$), there was no difference between Ground and Low sections ($P = 0.45$), but every other pairwise test showed significant differences ($P < 0.01$ to all) (Figure 2c).

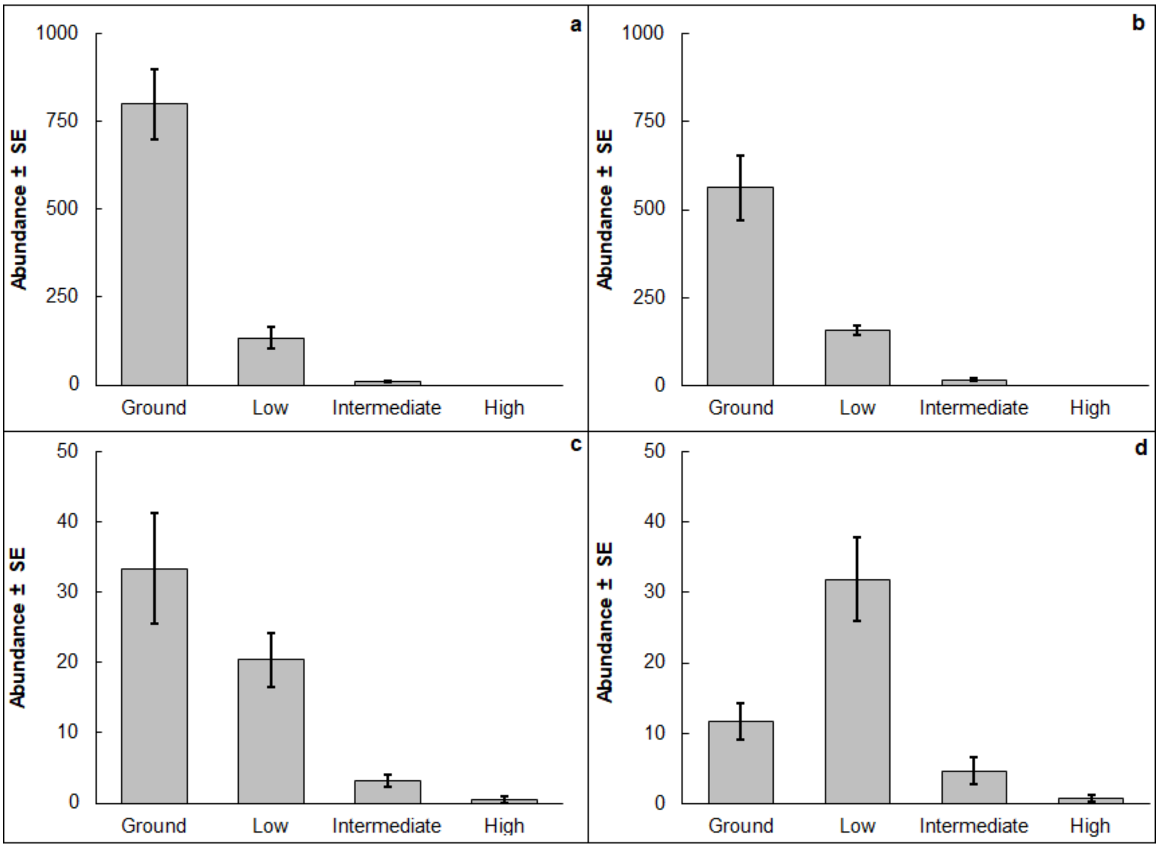
Contradictorily, abundance varied differently for phorids, wherein the Ground floor did not represent most of the catches; also, this was the only family represented at every sampled floor. The relative abundance of phorids across the sections was: 23.9% (Ground), 65.2% (Low), 9.5% (Intermediate) and 1.4% (High) (Table 1). For Phoridae ($F_{3,20} = 21.5$; $P < 0.01$), more flies were captured on the Low section than in all others ($P < 0.02$ for all), and while the abundance at Ground section did not differ from the Intermediate ($P = 0.17$), it did from the High section ($P < 0.01$), as exhibited in the Figure 2d.

Table 1 – List of species registered in the experiment, according to the level (and its approximate height from the ground, in meters) and the height treatment sections.

Families/species	levels and sections (highlighted)										
	0 (1.5)	3 (11)	6 (20)	9 (29)	12 (39)	15 (48)	18 (57)	21 (67)	24 (76)	27 (85)	Total
Calliphoridae	••••	•••	•••	•••	••	••					**5,658**
Chrysomya albiceps	••••	•••	•••	••	••	•					1,721
Chrysomya megacephala	••••	•••	•••	••	••	••					3,866
Chrysomya putoria	•										10
Cochliomyia hominivorax	•										1
Cochliomyia macellaria	••	•	•		•						17
Lucilia eximia	••										42
Lucilia sericata	•										1
Muscidae	••••	•••	•••	•••	••	••					**4,406**
Sarcophagidae	•••	••	••	••	••	•	•	•			**344**
Phoridae	••	•••	••	••	••	••	•	•	•	•	**293**
Total	**8,431**	**856**	**746**	**448**	**130**	**78**	**5**	**5**	**1**	**1**	**10,701**

Legend: 1-10 = •; 11-100 = • •; 101-1000 = • • •; 1001+ = • • • •

Figure 2 – Mean abundance ± SE of collected flies on each height section, according to the family: Calliphoridae (a), Muscidae (b), Sarcophagidae (c), and Phoridae (d).

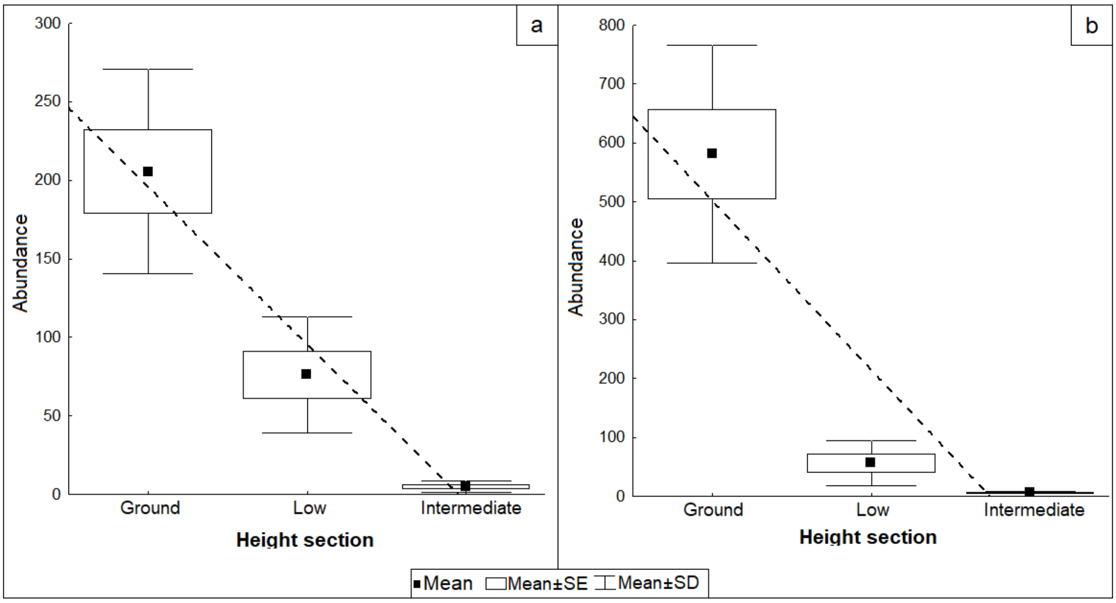


All families presented significant negative Spearman's rank correlations between abundance and height ($P < 0.01$ for all). For Calliphoridae (r = -0.95), Muscidae (r = -0.94) and Sarcophagidae (r = -0.90), the correlations were strong and for Phoridae (r = -0.66) the correlation was moderate, due to its different registered height stratification.

Considering the species of Calliphoridae, *Ch. putoria*, *Co. hominivorax*, *L. eximia* and *L. sericata* were only observed at the Ground, whilst *Ch. albiceps*, *Ch. megacephala* and *Co. macellaria* were also present at the Low and Intermediate sections. The vertical distribution of these three species followed the pattern described for the family, as they were more abundant on the Ground (71.7%, 90.2% and 64.7%, respectively), with inferior abundance on the Low (26.6%; 8.8%; 29.4%) and Intermediate sections (1.7%; 1.0%; 5.9%).

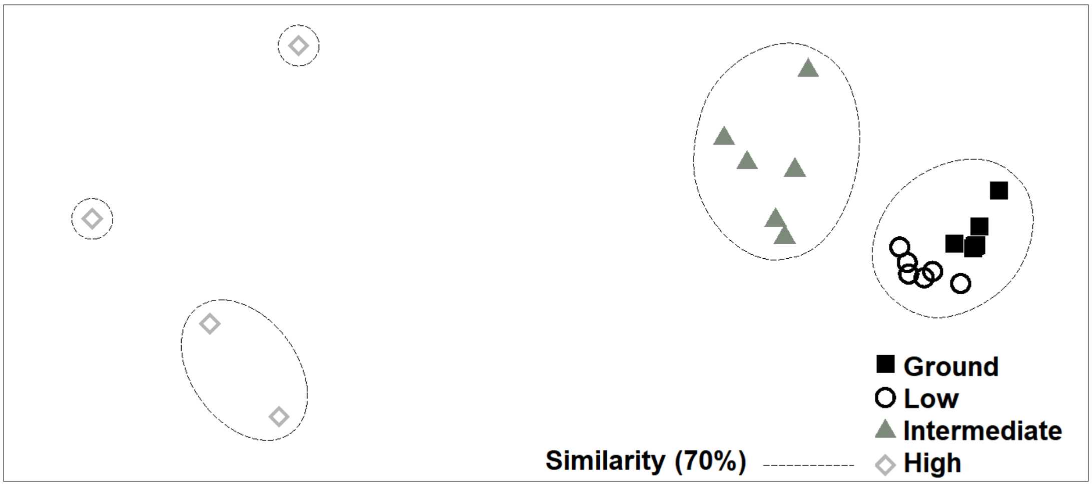
The register of flies closer to the ground was confirmed by the ANOVA, as in both *Ch. albiceps* ($F_{2,15} = 89.7$; $P < 0.01$) and *Ch. megacephala* ($F_{2,15} = 140.0$; $P < 0.01$), the majority of insects were collected on the Ground section when compared to all other ($P < 0.01$ for all) (Figure 3). Both tested species presented significant ($P < 0.01$) strong negative Spearman's rank correlations between abundance and height (*C. albiceps*: r = -0.93; *C. megacephala*: r = -0.95). Due to the low abundance, *Co. macellaria* was not tested to assess differences between height treatments.

Figure 3 – Mean Abundance ± SE and ± SD of *Chrysomya albiceps* (a) and *C. megacephala* (b), collected according to the height section. The tendency line (dashed) indicates the significant strong correlation between the variables.



The NMDS showed that most height sections had a tendency to form individual groups, with the exception of the High, which was represented by scattered marks, as a result of the low overall abundance associated with the absence of Calliphoridae and Muscidae (Figure 4). The ANOSIM endorsed the separation of groups with a calculated global R = 0.80. For the pairwise tests, the lowest value was registered between the Ground and Low sections (R = 0.86), that were grouped with a 70% similarity demarcation (Figure 4), while the remaining tests had a calculated value between R = 0.96 and R = 1.0.

Figure 4 – Nom-metric multidimensional scaling showing the formation of groups associated with the structure of the assemblage of collected flies from each height section of the buildings.



5 DISCUSSÃO

Our data confirm the hypothesis that necrophagous dipterans concentrate their flight activity at lower heights, a behavior endorsed by the fact that, under natural conditions, substrates for feeding and reproduction (mostly carrion) are available on – or close to – the ground. The observation of the flight demands the utilization of baits exposed to considerable heights, as flies do not discriminate baits exposed on the ground to those from a few centimeters to up to 1 meter (Bilaniuk and Beresford 2010).

Because adults of all families were not recorded in the highest treatments, it is possible to affirm that there is a simplification of the insect's assemblage at the High stratum. Calliphorids and muscids were not registered above the 15^{th} floor in any of the nine tested buildings. This fact both support preliminary data on the decrease in species richness as the height increases and, on the other hand, contradict previous registers of species of these four families equally registered at the high levels (Abdullah et al. 2016; Heo et al. 2017).

Curiously, Phoridae species do not follow a strict pattern of ground-dwelling behavior, as most specimens were collected on the Low section (11 to 29 m), whilst the Ground (1.5 m) and the Intermediate section (39 to 57 m) registered similar abundances. A combination of morphological, ecological, physiological and behavioral traits can explain this pattern. Scuttle flies have typically smaller bodies, high reproductive rates, and are often found as contaminant in forensic studies (as they are known as coffin flies), which may favor their highly mobile behavior. Thus, specimens of this family are able to explore higher substrates in detriment to lower ones, where the competition with other necrophagous adults is fiercer. There have been reports of phorid *Megaselia scalaris* (Loew, 1866) as early colonizer of substrates exposed to over 100 m (Heo et al. 2017), and as one of the most abundant in great elevations (Abdullah et al. 2016). However, both studies did not exclude passive transportation of insects and other human mediated dispersal processes, which is the basis for our study.

Since experimental data regarding flies' occurrence on heights superior to 10 m is scarce, it is hard to establish a possible maximum height for flight. For the conditions tested here, calliphorids and muscids reached a maximum of 48 m, sarcophagids attained 67 m, whilst phorids were captured in traps at the maximum tested height, ca. of 85 m.

The absence of Calliphoridae and Muscidae on higher treatments does not imply physical limitations to their flight, and may be a side effect of some experimental conditions. The search for a relatively small substrate (250 grams) might not compensate the energy expenditure in the long flight to reach it, under adverse, stressful conditions. Moreover, this absence can not be explained by morphological characteristics, as flies that have typically larger or smaller body sizes (sarcophagids and phorids, respectively), were found on traps exposed to higher treatments. It is likely that, had larger substrates been used, species of these families could have been registered on higher levels. Naturally, logistical, ethical and sanitary restraints to the use of the most common forensic model - pig carcasses - in buildings validate their replacement with animal baits, which have been proved to be excellent surrogate models (Farinha 2014).

To ratify that larger substrates might stimulate flies to reach greater heights, under conditions similar to those tested here, necrophagous flies from the families Calliphoridae, Muscidae, Sarcophagidae and Phoridae were found colonizing four decomposing human cadavers inside apartment buildings ranging from five to 15 floors in Malaysia (Abdullah et al. 2012; Syamsa et al. 2015). A similar register was made using a monkey carcass exposed on the 13^{th} floor (40 m) of a building, also in Malaysia (Abdullah et al. 2016). There is only one previous record of flies colonizing a cadaver inside an apartment building in Recife metropolitan region, and specimens of the four families were sampled; nevertheless, death occurred on the first floor so that no further inferences regarding height of flight can be achieved (Vasconcelos et al. 2014).

The presence of the invasive species *Chrysomya albiceps* and *C. megacephala* in urban settings is compatible with previous registers, and their dominance is commonly recorded in both urban and natural environments (Kavazos and Wallman 2012), probably caused by the high reproduction output associated with a rapid development cycle and a predatory behavior in its larval stage (Byrd and Castner 2009). Another important register is the occurrence *Cochliomyia hominivorax*, a species of medical and veterinary importance, that has been registered as causal agent of myiasis in Recife (Nascimento et al. 2005).

The experimental model chosen here, uninhabited buildings, proved to be efficient because of the great abundance of collected flies, and for the representativeness in the context of a verticalized urban center. Since the attractiveness of the baits to the flies is mediated by the release of volatile organic compounds (Dekeirsschieter et al. 2009), and its dissipation is influenced by the wind, the choice of buildings with large lateral openings, as well as the arrangement of the traps close to those openings provided more realistic settings.

Urban environments form an ideal scenario to investigate flight behavior of dipterans, due to their ubiquitous presence in cities and their extraordinary role in medical-legal entomology. Anthropogenic modification has been directly associated with the phenomenon of verticalization of cities. Consequently, population densities with abundant supply of organic matter available to dipterans are consolidated. Not surprisingly, large cities located in the tropics are associated with the highest rates of vector-borne diseases (Knudsen and Sloof 1992; Hollingsworth et al. 2015) and homicides (CCSPJP, 2017).

The ability of an insect to fly is a determining factor from an applied entomology perspective, by defining aspects as diverse as the vector capacity or the dispersion of a pathogen. In forensic entomology, characterization of cadaveric colonization often ignores the different competitive abilities intrinsic to each species (ability to detect the resource, flight capabilities, reproductive strategy, etc.). Surprisingly, crucial aspects of the behavior of synanthropic species – especially those of medical and legal importance – have been neglected. This is, to our knowledge, the first quali-quantitative study about the ability of necrophagous flies to reach substrates exposed to different heights in an urban center, and the data help to expand the knowledge about synanthropic necrophagous dipterans, with clear implications for health management and forensic procedures.

6 CONCLUSÃO

A partir desta tese, espera-se ter contribuído com o conhecimento existente sobre moscas necrófagas, em especial as da família Calliphoridae. Como resultados principais desta tese, podemos citar:

- A maioria dos califorídeos, muscídeos e sarcofagídeos são encontrados em maior abundância mais próximos ao solo;
- Os phorídeos, no entanto, apresentam um padrão de voo com maior abundância em níveis acima do solo, possivelmente para evitar competição pelo substrato com as outras espécies;
- Nas condições testadas, califorídeos e muscídeos foram coletados entre o térreo e o 15° andar, enquanto sarcophagídeos e phorídeos até o 21° e 27°, respectivamente;
- Califorídeos merecem destaque pela abundância das espécies invasoras *Chrysomya albiceps* e *C. megacephala*, e também pelo registro de *Cochliomyia hominivorax*, espécie causadora de miíases.
- As espécies de califorídeos *Cochliomyia macellaria* e *Chrysomya albiceps* são mais abundantes nos horários mais quentes e secos do dia;
- A ausência de voo noturno fortalece a hipótese de que este comportamento quando registrado, deve ser considerado acidental.
- Várias espécies de moscas necrófagas são capazes de colonizar um cadáver humano ao mesmo tempo*;*
- *Chrysomya albiceps* e *C. megacephala*, colonizam cadáveres humanos rápido e eficientemente, tendo as larvas sido depositadas em um intervalo temporal curto, evidenciado pela idade semelhante dos indivíduos coletados;
- O registro de *Fannia pusio* (Fanniidae) é importante, uma vez há apenas registros anedotais da sua presença em cadáveres humanos. Além disso, a sua potencial utilização forense é comparável às de outras famílias, indicado pelo intervalo pós-morte semelhante ao gerado pelas outras espécies;

- Com base na evidência entomológica subsidiada pela presença destas três espécies, estima-se a morte entre 28 a 36 horas antes do descobrimento do corpo.

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APÊNDICE A - ARTIGO PUBLICADO NO PERIÓDICO JOURNAL OF ARID ENVIRONMENTS

Do native and invasive blow fly (Diptera: Calliphoridae) species differ in their preferential time of flight? Empirical evidence from a seasonally dry tropical forest

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Abstract

Since its establishment in the Americas, the invasive species *Chrysomya albiceps* has been displacing native species such as *Cochliomyia macellaria*. Behavioural traits associated with this phenomenon are poorly understood, particularly in semiarid environments, as in the *Caatinga*, the largest seasonally dry tropical forest in the world, endemic to Brazil, where resource availability is critical. Under hot and dry conditions, differences in flight activity may influence competitive abilities. We investigated the preferential time of flight of *C. albiceps* and *C. macellaria* in a fragment of *Caatinga*, based on the capture of adults in baited traps. Four diurnal treatments were used: 05:30 h to 08:30 h (early morning), 8:30 h to 11:30 h (late morning), 11:30 h to 14:30 h (early afternoon) and 14:30 h to 17:30 h (late afternoon), plus collections from 17:30 h to 05:30 h of the following day (night time). The results revealed that *C. macellaria* was almost three times more abundant than *C. albiceps* and that both species were least captured in the early morning and increasingly so during the day until the latter treatment. Curiously, no insect was collected in nocturnal samples. Contradictorily to our initial hypothesis, flies were mostly captured in the hottest and driest periods of the day. The similarity in flight temporal activity between the two species suggests that they may arrive at (and colonize) a substrate at the same time, with important implications for carrion ecology and forensic entomology.

Key words: *Caatinga*; semiarid; behaviour; flight activity; stressful conditions; *Chrysomya albiceps*, *Cochliomyia macellaria.*

Blow flies (Diptera: Calliphoridae) have a remarkable ability to detect, reach and colonize decomposing animal substrates, which enables them to dominate decaying, ephemeral resources (Byrd & Castner 2009). High biotic potential combined with adaptation to different environmental conditions contribute to the establishment of invasive species, such as those of the genus *Chrysomya* Robineau-Desvoidy, 1830. Species of this genus, such as *C. albiceps* (Wiedemann, 1819) are now widely distributed in South, Central and North America and impact negatively the reproduction and survival of native species, such as *Cochliomyia macellaria* (Fabricius, 1775) (Faria et al., 1999). Coexistence between native and invasive species is largely mediated by a series of traits, of which flight and dispersal are amongst the most important.

Environmental characteristics strongly influence blow flies' activity which may be accelerated or deterred according to thermal conditions (George et al. 2013). Blow flies have been registered flying under controlled temperatures ranging from 9 °C to approximately 40 °C (Nicholson 1934; Nuorteva 1966). From the minimum threshold onwards, flight is almost linearly stimulated until a peak, from which it declines until a critical maximum temperature is reached, after which no movement is recorded (Nicholson 1934). Conversely, under natural conditions, flight behaviour might exhibit different patterns (Paraluppi & Castellón 1993; Soares & Vasconcelos 2016). This phenomenon, however, is still poorly investigated in the field, especially in semiarid areas.

The largest and most diverse semiarid forest in the New World is the *Caatinga*, a domain restricted to the Brazilian territory, characterized by irregular rainfall distribution, that concentrates most of the rain in a few months a year (Silva et al. 2017). Information on blow fly flight activity patterns in the *Caatinga* has only recently been studied (Oliveira & Vasconcelos 2018). Because of their forensic relevance, data about diurnal and nocturnal activity including preferential time of flight enhances their potential utilization on criminal investigations (Byrd & Castner 2009).

The objective of this study was to describe the temporal flight pattern of *Chrysomya albiceps* and *C. macellaria* in a *Caatinga* fragment, with emphasis on their diurnal flight. We hypothesize that, due to the harsh abiotic characteristics, both species prefer to fly under milder abiotic conditions found in the earlier periods of the day.

The experiments were carried out in a fragment of dry forest (*ca.* 7 ha) in the municipality of Afogados da Ingazeira (7°44'14.8"S; 37°35'12.2"W), Northeastern Brazil

(Figure 1A). The area has two well-defined seasons, rainy (from January to May) and dry (from June to December), with annual rainfall around 592 mm, mean temperature of 25 °C and mean relative air humidity of 55% (Köppen's Bsh – semiarid hot). The landscape is flattened; soil is mostly shallow, stony, or even nonexistent in areas with rocky outcrops. Vegetation is composed by shrubs and irregularly distributed trees, with abundance of native species of Anacardiaceae, Bromeliaceae, Cactaceae, Fabaceae and Rhamnaceae. Intense solar radiation reaches most of the ground through scattered clearings in the forest.

The experiment comprised three field expeditions, from January 2016 to February 2017. Flies were captured in suspended traps (Figure 1B), baited with 150 g of previously decomposed bovine spleen, described elsewhere (Oliveira et al. 2016). Collected flies were stored in 70% ethanol until identification with the taxonomic keys found in Carvalho & Mello-Patiu (2008).

To assess flight activity, evidenced by the capture of flying insects, sampling was performed in four separate, non-overlapping, treatments: 05:30 h to 08:30 h (early morning - EM), 8:30 h to 11:30 h (late morning - LM), 11:30 h to 14:30 h (early afternoon - EA) and 14:30 h to 17:30 h (late afternoon - LA). Additionally, we investigated nocturnal flight by sampling from 17:30 h to 05:30 h of the following day (night time - NT). Temperature and relative humidity of the air were measured *in loco*, and rainfall data were obtained from the nearest meteorological station in the municipality. In each field expedition, sampling was carried out through four consecutive days, and 72 replicates of each one of the five time-treatments were tested, so that 360 observational units (traps) were considered for the analysis.

To infer about differences in insect abundance between temporal treatments, data were submitted to either ANOVA or Kruskal-Wallis test; when only a pair of parameters were analyzed, such as the abundance of both species, and their relative abundance between time treatments, the differences were assessed through a *t*-test or the non-parametric Wilcoxon-Mann-Whitney test. Linear correlations between insect abundance, temperature and relative humidity were also calculated. All tests and graphics were made on Microsoft Office® 2016 suite and Statistica® 7.0, Primer® 6.0 and BioEstat® 5.3 software, with significance level of 5%.

When data from all 360 traps were compiled, a total of 4,891 flies were collected. Most individuals were *Cochliomyia macellaria*, which represented almost three quarters of total (73.6%), while the other 26.4% were *Chrysomya albiceps* (T = 2.76; d. f. = 142; P = 0.006). Regarding the period of the day, no calliphorids were collected on the traps exposed in the nocturnal period. During the diurnal treatments, the abundance of both species was lower in the initial hours, gradually increasing throughout the day.

For *C. albiceps*, the EM treatment (05:30 h to 08:30 h) accounted for 16.2% of the abundance, followed by LM (08:30 h to 11:30 h) with 16.7%, EA (11:30 h to 14:30 h) with 25.2% and LA (14:30 h to 17:30 h) with 41.9%. For *C. macellaria* the values were: 14.5% in EM, 18.8% in LM, 25.9% in EA and 40.8% in LA. For both *C. albiceps* ($F_{3;284} = 8.62$; $P < 0.0001$) (Figure 2A), and *C. macellaria* ($F_{3;284} = 9.33$; $P < 0.0001$) (Figure 2B), flies were less abundant at the earliest treatment EM than those caught in the latter treatments EA and LA ($P < 0.01$ for both), and those from the second treatment LM were less abundant than those from LA ($P < 0.05$ for both). Comparison of the proportion of insect species at each temporal treatment reveled similarity in the pattern of flight activity ($P > 0.05$ for all tests).

Overall mean temperature during the experiment was 28.6 °C and the RH was 53%, but there was an increase in temperature and a decrease in humidity throughout the daytime treatments until 14:30, after which an inversion of the pattern occurred, with a decrease in temperature and increase in RH (Figure 3). We recorded the lowest mean temperature and the highest relative humidity at 05:30 (21.1 °C and 76%, respectively), whilst the hotter and driest conditions were registered at 14:30 (34.2 °C and 41%). As typical from semiarid environments, abiotic factors exhibited wide amplitude on a single day. For instance, temperature rose from 17.0 °C to 35.8 °C and RH dropped from 70% to 15% from the morning to the afternoon. Because of the inversion on temperature and RH, no direct linear correlation between abundance and these variables was accounted for in both species ($P > 0.05$ for both).

In this study we provide experimental evidence on the temporal pattern of flight activity of two of the most important species of Diptera in forensic entomology in the Neotropical region. Both *C. albiceps* and *C. macellaria* are well stablished in *Caatinga* and tend to be dominant over other Calliphoridae species (Vasconcelos et al., 2016), as well as in other environments such as rainforest (Carmo et al. 2017), coastal environments (Barbosa et al. 2017) and urban areas (Paraluppi & Castellón 1993). The data presented here reveal similarities in diurnal temporal pattern of flight of both species, with lower abundance in earlier hours of the day, with a peak in later hottest and driest hours, which contradicts the initial hypothesis.

Our findings diverge from those of Soares & Vasconcelos (2016) in an Neotropical Atlantic forest fragment, according to which blow flies, including *C. albiceps*, are most active at early morning, when temperature is cooler and the air is more humid. In another discordant result, Paraluppi & Castellón (1993) reported that neither *C. albiceps* or *C. macellaria* presented any preferential time of flight, as both species were captured in similar abundance throughout the day, without significant influence of hour of collection, temperature or relative air humidity. So far, data from Neotropical region suggest that local environment characteristics may affect similarly different species, irrespective of their origin.

Data from subtropical and temperate regions point out that in cold places or during cold seasons, blow flies tend to fly preferentially around midday, and in hot environments and/or under hot seasons, they have a bimodal distribution, with peaks before and after the hottest hours, during which they seem to seek refuge and avoid flying (Das et al. 1978), which is also different from the pattern registered here. The influence of abiotic factors, such as temperature, relative humidity, solar irradiation and luminosity on insect flight is inconsistent and seems to vary according to local conditions as shown here and on other studies from Neotropical region (Paraluppi & Castellón 1993; Soares & Vasconcelos 2016; Oliveira & Vasconcelos 2018).

Field-generated data on insect flight contribute for the prediction, for example, of which species fly under a broader range of conditions and indicate competitive advantage in the exploitation of ephemeral resources. In addition to that, is it known that calliphorids flight may not be entirely related to instantaneous temperature, but it changes when the specimens are analyzed under a steady and constant conditions, as in controlled experiments, or under a varying temperature, as occur in field trials (Nicholson 1934).

Under these circumstances, the present work shows that at least for the *Caatinga*, the flies are more active (evidenced by the highest capture) in late hours of the diurnal period, which registered the highest temperatures and the lowest relative air humidity, suggesting that flight activity might be influenced by cumulative effects, as proposed by Digby (1958). It is important to stress out that although dry tropical forests are considered a harsh, inhospitable environment – especially in the dry season – the high temperatures and low humidity does not reduce flight activity of blow flies.

Blow flies, especially those from the genus *Chrysomya*, are capable to withstand a broad range of temperature in all stages of the life cycle (Richards et al. 2009). Mechanisms that explain the adaptation of blow flies to thrive (and fly) on hot and dry environments include their ability to benefit from the high temperature during its larval stage, accelerating their development (Richards et al. 2009). In addition to that, adult calliphorids are protected by a thick cuticule that reflects light (Nuorteva 1966), which help them resist to higher external temperatures when compared to other fly species.

Nocturnal flight of blow flies is a contradiction broadly reported on literature. Our findings echo those of Stamper et al. (2009), who did not observe nocturnal flight. However, Soares & Vasconcelos (2016) detected minimum nocturnal flight of Calliphoridae species, such as *Mesembrinella bicolor* (Fabricius, 1805), as opposed to *C. albiceps* and *C. macellaria*. Although nocturnal flight in the *Caatinga* has been observed (Oliveira & Vasconcelos 2018), it seems to be an accidental record – with an extremely low abundance – as registered elsewhere (Stamper et al. 2009). Possible explanations for this conflict is the presence of artificial light in other studies, which revealed that in the absence of light, flies prefer to walk to the substrate instead of fly direct to it (George et al. 2013), which reduce the probability of capture in suspended traps.

The combined impact of behavioural features (e.g., flight, resource location) with physiological parameters (e.g., reproductive capacity, predatory habit) determines the dynamics in coexistence of native and invasive species. Flight activity is directly related to the ability to exploit ephemeral resources, particularly under stressful conditions, with implications for conservation biology and forensic entomology. Although the register of homicides are clearly sub notified in South America, lethal crimes have risen dramatically in the past few years in cities located in the Brazilian semi-arid region (Waiselfisz 2016). Because data on blow fly behaviour subsidize the estimation of minimum post-mortem interval, it is likely that the species tested here would not differ on the arrival at a cadaver irrespective of the time of the day, based on the similarity in flight patterns. Forensic experts should take that into account in the sampling entomological evidence. The complexity of the impact of flight behaviour on such basic and applied issues is only beginning to be unveiled.

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FIGURE LEGENDS

Figure 1 – Map of Brazil, with the approximate locality where the experiment was carried out (a), and the trap used during the experiment (b).

Figure 2 – Mean abundance ± standard error and ± 95% of the confidence interval of *Chrysomya albiceps* (a) and *Cochliomyia macellaria* (b) sampled during each temporal treatment.

Figure 3 – Abundance of collected *Chrysomya albiceps* and *Cochilyomyia macellaria* according the temporal treatments (left Y axis) and corresponding mean temperature (°C), relative humidity (%) (right Y axis).

FIGURES

Figure 1

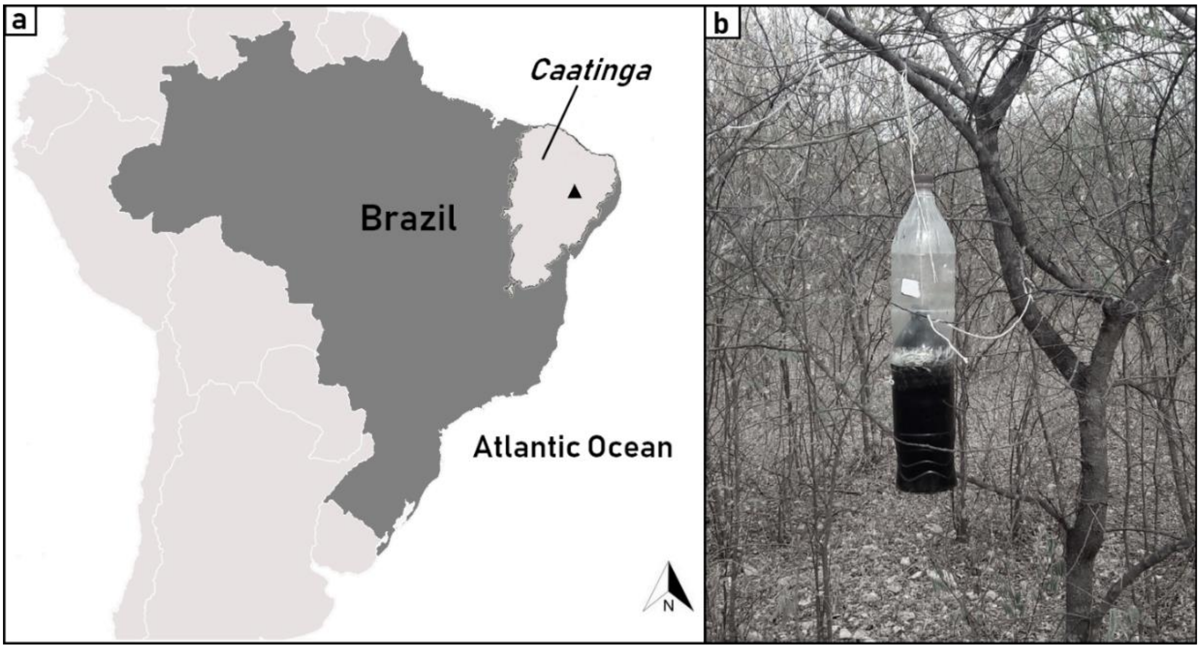
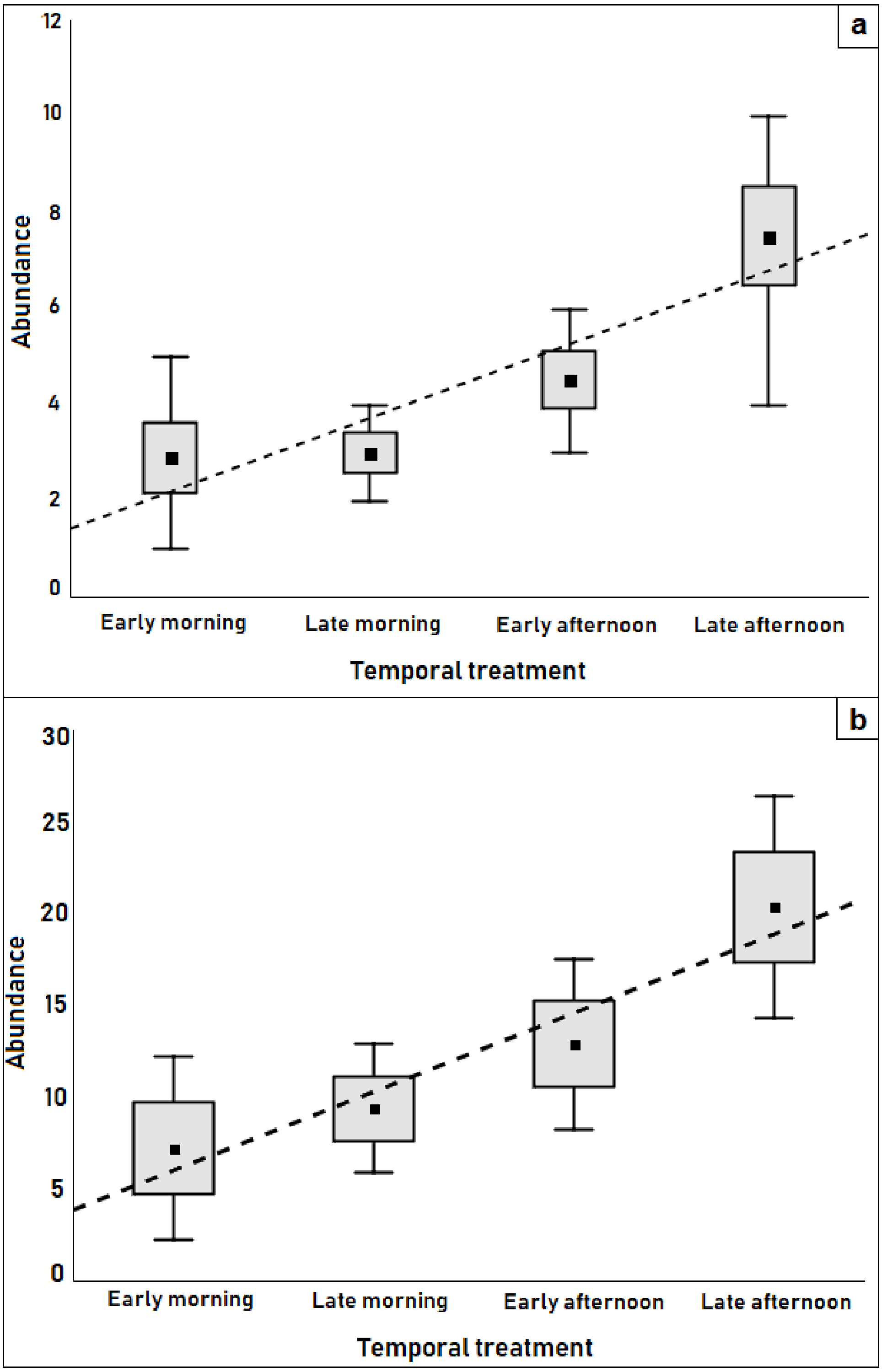


Figure 2


a
Abundance
12
10
8
6
4
2
0
Early morning
Late morning
Early afternoon
Late afternoon
Temporal treatment
b
Abundance
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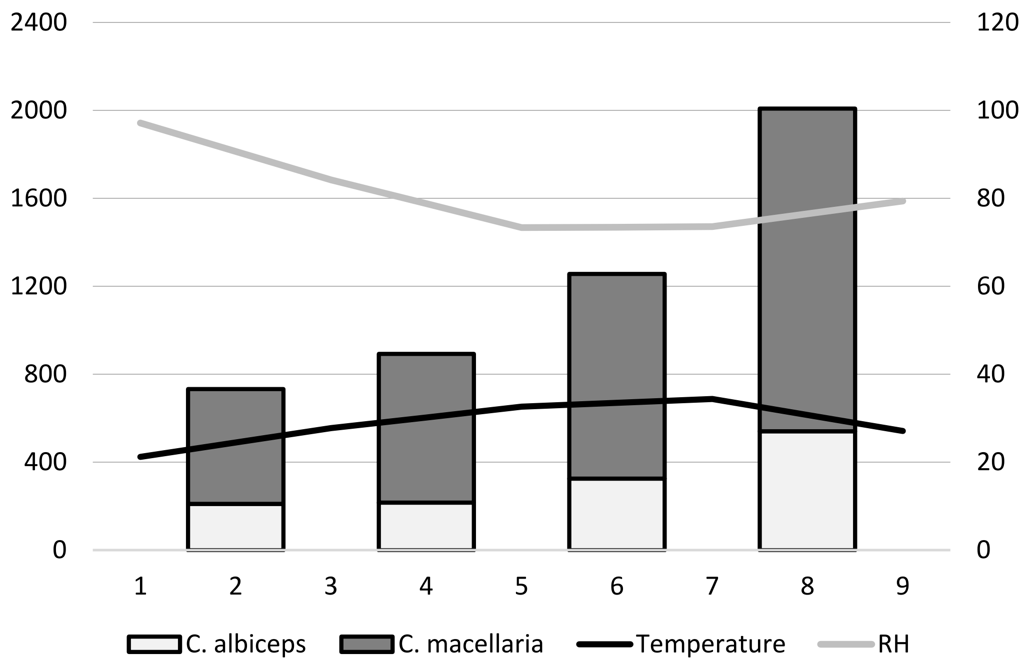
Early morning
Late morning
Early afternoon
Late afternoon
Temporal treatment

Figure 3



APÊNDICE B – ARTIGO PUBLICADO NO PERIÓDICO AUSTRALIAN JOURNAL OF FORENSIC SCIENCES

Entomological evidence in a case of a suicide victim by hanging: first collaboration between entomologists and forensic police in north-eastern Brazil

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Abstract

Peculiarities associated with the site and type of death impact the interpretation of entomological evidence in forensic investigations. We investigated the diversity and time to emergence of necrophagous species associated with a hanged cadaver in a city exposed to high levels of homicide in Brazil. Six species of four families of Diptera (Calliphoridae, Fanniidae, Muscidae and Sarcophagidae) colonized the corpse, of which *Chrysomya albiceps* (Calliphoridae) corresponded to 97% of all emerged adults. We provide supporting evidence for the record of *Fannia pusio* (Fanniidae) and for *Hydrotaea aenescens* (Muscidae) as colonizers of human cadavers. The time elapsed from collection of larvae until emergence of adults varied from 8 days (*Chrysomya megacephala*) until 19 days for *F. pusio*. A higher density of maggots occurred on the soil immediately below the cadaver when compared with the body. The use of time of development of both *C. albiceps* and *C. megacephala* provided similar estimations of the minimum post-mortem interval and suggested that death occurred approximately 36 hours prior to the discovery of the body. This case provides a novel collaboration between entomologists and forensic police in north-eastern Brazil and reinforces the importance of Fanniidae as a forensically important group in medico-legal investigations.

Key words: Forensic entomology; Calliphoridae; post-mortem interval; Muscidae; Fanniidae

INTRODUCTION

A key assignment of forensic entomologists is to interpret evidence that may help to elucidate the time, conditions and cause of death. This is particularly urgent in areas exposed to endemic violence, such as north-eastern Brazil[1], where an alarming number of homicides are unsolved[2]. This situation has prompted police and law enforcement professionals to make use of multidisciplinary tools, such as forensic entomology. So far, necrophagous species of the families Calliphoridae and Sarcophagidae (Diptera) have been the most frequently used taxa in criminal investigations, which tends to overlook the reliability of data provided by other families.

The effective use of forensic entomology to estimate the minimum post-mortem interval (minPMI), corpse transfer and the presence of illicit substances on the body[3] requires realistic bionomical data about insect species that colonize and complete their life cycle on human bodies. If not taken into account, particular issues associated with the site and the type of death may compromise the interpretation of field-generated data. Suspended bodies, for instance, are thought to harbor a lower diversity of insects that tend to develop at a slower rate when compared with corpses that remain in full contact with the soil, although this phenomenon has been investigated using mostly pig carcasses[4,5]. Considering that hanging is one of the most common methods of suicide worldwide and has an extremely high fatality rate of over 70%,[6] it is surprising that data on insects associated with suspended corpses is mostly anecdotal.

Because information on the patterns of corpse colonization by insects helps in directing evidence collection and interpretation, data obtained *in loco* are crucial: the more species are reported as colonizers of cadavers, the more realistic will be the databases available to forensic entomologists in a particular area. In this study, we describe the entomological fauna associated with a hanged, half-suspended corpse, focusing on the diversity of species of Diptera that use the cadaver as a resource for the development of the immature stages in a peri-urban area in north-eastern Brazil. We also aimed to compare the diversity of species present on the cadaver with that on the surrounding soil, considering that most dipteran species tend to disperse prior to pupation[7]. Finally, this study aimed to compare the time to emergence of the most common species, in a context related to the potential estimation of the minPMI. This case of a suicide victim consists of the first collaboration between entomologists and police professionals in north-eastern

Brazil, where the elevated rates of homicide provide a favourable scenario for the strengthening of forensic entomology

METHODS

Characteristics of the area and of the body

On 26 January 2016, at 8:10 pm, the body of a 46-year-old male (approximately 80 kg) dressed in t-shirt and Bermuda shorts was found in incomplete suspension (partially hanged) tied up to a tree by a piece of cotton clothing (Figure 1(a)). For ethical reasons, the identity of the deceased was kept anonymous throughout this study. The body was found in a peri-urban area in the municipality of Jaboatão dos Guararapes (8°10'38.50" S; 34°56'52.53" W, altitude 75 m, est. pop. 691,000) located in the Metropolitan Region of Recife, capital of Pernambuco State, north-eastern Brazil. The area, surrounded by native shrubs and sparsely distributed trees, was located near the BR-101 Southern highway and is exposed to heavy traffic. Mean temperature in the area in the period was 26.5°C and there was no rainfall for 15 days prior the discovery of the body.

Following an anonymous phone call, the police arrived and isolated the area, after which the investigators collected the evidence. Forensic examination did not find any evidence of struggle, defensive wounds or signs of violence on the body so that death was attributed to suicide by asphyxia due to hanging. As this was a case of self-inflicted death with no criminal nature, no further analyses (e.g. toxicology) were performed. The body was at the end of the bloated stage with some parts (e.g. face) showing some characteristics of the active decay stage[8]. Within 30 min of the discovery of the body, the police investigators called the senior entomologist at the city's university to supervise the collection of entomological evidence, given that only the forensic police are entitled to have access to the scene of death

Insects associated with the cadaver

After thorough inspection of the body and its surroundings, larvae were collected using soft forceps, and soil samples were also taken to the laboratory. To obtain information about the age and spatial distribution of larvae, samples were taken from the following sites (Figure 1(b)): (i) head and neck; (ii) upper body, including the clothes; (iii) soil immediately below the corpse and in a radius from the knees up to 0.5 m; and (iv) surrounding soil (ca. 0.5 to 1.5 m from the corpse). Due to logistical and safety reasons, sampling was intensive so that a standardized sampling time of 15 min on each site was performed. Soil was sampled because it was expected that larvae collected distant from the cadaver would be at the post-feeding stage, when most species tend to disperse prior to pupation.

In order to estimate the age of the larvae on the cadaver and to identify the colonizing species, all sampled larvae were reared in the laboratory, at 26 ± 2°C, in plastic containers with minced beef as food and sawdust as substrate for pupation. Insects were observed every 12 h until emergence of all adults, which were immediately removed from the containers to avoid a second generation, and then identified according to specific taxonomic keys[9–12]. Differences in the abundance of the emerged adults and in the spatial distribution of larvae were compared among species using Chi-square tests. The time from collection until emergence of adults among the sampling sites for each species was compared using the analysis of variance of Kruskal-Wallis with a posteriori Student-Newman-Keuls tests. All analyses were performed using the BioStat 5.0 and Statistica 7.0 softwares at a 5% significance level.

RESULTS AND DISCUSSION

A total of 5,600 adult dipterans emerged from the larvae collected on the cadaver. Six species were registered: *Chrysomya albiceps*, *Chrysomya megacephala*, *Hemilucilia segmentaria* (Calliphoridae), *Hydrotaeae aenescens* (Muscidae), *Fannia pusio* (Fanniidae) and *Peckia* sp. (Sarcophagidae). Of these, *C. albiceps* was clearly the most abundant species and corresponded to 97.1% of all emerged specimens, followed by F. pusio and *C. megacephala* (each with 1.4% of individuals). Only two specimens of H. aenescens, one *H. segmentaria* and one *Peckia* sp. emerged. Comparatively, the soil below the cadaver had the highest diversity (six species) and abundance of larvae (Table 1).

The dominance of the invasive species *C. albiceps* registered in this study is in accordance with surveys performed on carcasses and cadavers in several countries[13,14]. In a recent study performed in north-eastern Brazil, *C. albiceps* and *C. megacephala* comprised 65.0% and 19.0%, respectively, of all colonizing insects in a cadaver located in a first floor flat with limited access to flies[15]. Several features of *C. albiceps* justify its geographical distribution and competitive success: its ability to colonize a wide range of substrates, short life cycle, high fecundity, and aggressive behaviour of the larvae, which includes predation, that may displace native species in resource colonization[16].

Chrysomya megacephala is an invasive species that has been reported as a colonizer of human corpses in urban and forested areas in South America[14,17]. In an experiment performed in Thailand, *C. megacephala* was the dominant species on hanging pig carcasses dressed in clothes to simulate human corpses[18]. This species is highly efficient in locating decomposing resources and has been collected on carcasses just minutes after death[19].

Hemilucilia segmentaria is described as a typically asynanthropic species that inhabits forested areas[20], but this assumption must be taken with care, because the corpse was found in a peri-urban area surrounded (at a distance of ca. 0.5 km) by factories and intense traffic on the busiest highway of the region. The forensic relevance of the species has been strengthened by a case of estimation of PMI in the Amazon using data on its lifecycle[21].

Hydrotaea aenescens is a widespread species native to the Neotropical region common in urban environments[22]. Its association with human cadavers is rare, and it is mostly documented during the active decay stage of decomposition[7] and with buried corpses[23,24]. Larvae are also found in body exudates that soak the soil beneath remains[7], as observed in this study, in which the specimens were collected from the soil below the cadaver (Table 1). In a recent review, Grzywacz et al.[25] discuss the need for a more realistic examination of the forensic potential of species of Muscidae, including *H. aenescens*, and point out inconsistencies in taxonomical identification of species of *Hydrotaea* worldwide.

Reports of *F. pusio* developing on cadavers have been scant and circumstantial[26]. This is, to our knowledge, the first in-depth description of its occurrence on human cadavers, associated with information on its developmental time under a forensic approach. With over 285 species, *Fannia* is the most diverse genus of the family Fanniidae, and occurs in all zoogeographic regions of the world, most of which have been described in the Holarctic region[27]. Although *Fannia canicularis*, *F. scalaris* and *F. pusio* are widely distributed, most species of Fanniidae are restricted to biogeographic regions, such as the Holarctic, Australia, New Zealand, Africa and South America[28]. *Fannia* species have been increasingly studied in forensic entomology, as they are found in autopsy rooms[14] and have been used as evidence of child neglect in Germany[29]. *Fannia scalaris* was found in association with a corpse in advanced decay stage in Central Italy[30] and, recently, *F. trimaculata* was reported as an early colonizer of a human cadaver in an urban setting in South America[15].

Although hanging is one of the most common methods of suicide worldwide[6], there is remarkably little knowledge on the patterns of cadaver colonization by insects on hanging corpses. Naturally, due to ethical and logistical reasons, there are no experimental, replicated data on human corpses, so that extrapolation is inferred from trials performed using pig carcasses[15]. Only two cases have been described in Brazil. In the case of a 35-year-old male found in an urban area, maggot masses were concentrated on the lesion on the neck of the deceased that was colonized exclusively by *C. albiceps*[14]. In a case of incomplete hanging in Central Amazon, a few adults of an asynanthropic species *(H. segmentaria*) emerged[20]. A higher diversity is reported here, despite limited exposure time in the field. In contrast to records of aggregation of larval masses on the neck of victims of hanging[14], no concentration of larvae was found on the neck in this case, probably because there was no laceration of the tissues due to the soft material used.

The adults emerged between 7 and 25 days post-collection of the larvae in the field (Table 2). The mean (± SD) times to emergence were the following: *C. megacephala* = 8.6 ± 0.77 days; *C. albiceps* = 10.6 ± 1.27 days; *F. pusio* = 17.8 ± 1.60 days. However, the emergence of *C. megacephala* and *F. pusio* were concentrated on a slightly narrower interval (7 to 11 and 15 to 20 days, respectively) when compared with *C. albiceps* (8 to 14 days) (Figure 2).

For comparison of the validity of *C. albiceps* and *C. megacephala* in the estimation of the minimum post-mortem interval, we used combined data on the life cycle of *C. albiceps* reared at 26°C, which takes approximately 11.8 days from egg until adult[31,32]. The bionomical data obtained here (ca. 10.6 days from collection of larvae in the second instar until emergence of adult) lead to an average 1.2 day interval between oviposition and sampling, which is in agreement with the duration of the egg (11 h) and first instar (16 h) stages[31]. By applying bionomical data of *C. megacephala* reared in similar conditions, total life cycle would be ca. 10.0 days[33], which provides an interval of 1.5 days between oviposition and collection, given that adults took an average of 8.5 days to emerge.

Although PMI was not estimated officially, the entomological evidence from two different calliphorid species allowed for an accurate prediction. Using the time to emergence of insects collected on the corpse, oviposition was supposed to have occurred between 28 h and 36 h prior to the discovery of the body. Based on the experience of the police personnel involved, it is inferred that death occurred at night, especially given that the peri-urban area may be accessible to passers-by during the day. Considering that nocturnal oviposition is rare in local conditions[34] an 8–12 h interval is likely to have occurred between death and oviposition. The combined effect of low nocturnal activity of blowflies in the Neotropical region[34], the lack of lacerations in the neck, the presence of clothes and the fact that the corpse was partially suspended could have resulted in delayed cadaver colonization in the field.

Interestingly, the time to emergence of larvae collected on the surrounding soil did not differ significantly from those larvae collected on the body ($P > 0.05$), which indicates that larvae sampled on different sites were approximately at the same age. Therefore, the assumption that larvae on the surrounding soil would be older than those from the body as a result of post-feeding dispersal behaviour[7] should be pondered to prevent contradictory information for PMI estimation[35]. In this case, the fact that the knees were in contact with the soil may have allowed larvae to disperse from the body, besides falling off directly to the soil.

Observations on hanging pig carcasses have revealed a much slower decomposition rate than those in contact with the soil, and this delay results from the lower diversity and quantity of insects present on suspended bodies due to restricted access to several species (e.g. beetles) and the loss of maggots that fall from the cadaver[5]. Accordingly, Shalaby et al.[4] reported that a significant site of insect activity was observed on the surface of the soil immediately under the hanging carcasses. Thus, our observations support the idea that soil samples from the vicinity of cadavers supply a reliable source of entomological evidence, as demonstrated here, provided that extraneous variables are incorporated into the models of PMI estimation.

This is, to our knowledge, the first *in loco* study of entomological evidence collected on a hanging cadaver in a large city in South America, and the fact that cities in north-eastern Brazil harbor some of the highest homicide rates in the world confers a particular relevance to this case. Differences in the time of colonization based on two species were slight (1.2 days for *C. albiceps* and 1.5 days for *C. megacephala*). The unique features associated with the decomposition of a half-suspended cadaver must be taken into account when choosing the best surrogates for the estimation of the PMI. From that perspective, data on the development of *C. megacephala* would be more useful in the estimation of PMI than those on *C. albiceps*, in this case, due their narrower 'window' of emergence – which would generate more precise predictions of larval age – and therefore the PMI[36]. The invasive nature of both *Chrysomya* species and their competitive ability to locate and colonize ephemeral resources tends to lead to the neglect of other species in the estimation of minimum post-mortem.

The register of *F. pusio* as a colonizer of cadavers and the preliminary data on the development time of this species presented here help to substantiate the forensic importance of Fanniidae and highlight the need for detailed bionomical studies. Combined with ecological and behavioural data (i.e. post-feeding dispersal), the diversity and bionomics of the species reported here aid in the understanding of decomposition processes associated with specific types of death, such as hanging. Despite the shocking rates of homicide in north-eastern Brazil, the tortuous mechanisms in the legal system still hinder the use of scientifically proven evidence, given that no court cases involving forensic entomology have ever taken place in the region. This scenario appears to be in common with other countries, such as Australia (major differences in homicide rates notwithstanding), where, according to Archer and Wallman[37], the relatively isolated nature of most practitioners tends to slow down the progress of this field. As stressed by Robertson[38], forensic entomology can only advance if investigators pay proper regard to the contribution of forensic scientists, in team work. The current case illustrates an embryonic collaboration between university entomologists and forensic police and contributes to the development of forensic entomology in the region.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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Table 1. Diversity and abundance of species of Diptera associated with a hanged cadaver in a peri-urban area in Recife, Brazil.

Family / Species	Head and neck	Upper body	Soil below	Surrounding soil
Calliphoridae				
Chrysomya albiceps	****	***	*****	***
Chrysomya megacephala	**	**	*	**

Hemilucilia segmentaria	-	-	*	-
Muscidae				
Ophyra aenescens	-	-	*	-
Fanniidae				
Fannia pusio	**	-	**	**
Sarcophagidae				
Peckia sp.	-	-	*	-

Legend: □= < 10; □□= 10 to 200; □□□= 201 to 1,000; □□□□= 1,001 to 2,000; □□□□□= > 2,000

Table 2. Mean time to emergence (days ± SD) of dipterans collected on the corpse and surroundings, according to the site of collection. Mean times to emergence did not vary significantly across different sampling sites for the three species ($P > 0.05$).

Species	Head and neck	Upper body	Soil below	Surrounding soil
Chrysomya albiceps	10.3 ± 0.68	10.2 ± 0.64	10.7 ± 1.36	11.8 ± 0.93
	Interval of time to emergence = 8 to 14 days Mode = 10 days (46.80% of all specimens)			
Chrysomya megacephala	8.7 ± 0.87	8.6 ± 0.62	7.8 ± 0.50	8.4 ± 0.77
	Interval of time to emergence = 7 to 11 days Mode = 8 days (55.13% of all specimens)			
Fannia pusio	17.7 ± 1.11	-	17.4 ± 1.89	18.3 ± 1.09
	Interval of time to emergence = 15 to 20 days Mode = 19 days (46.83% of all specimens)			

Figure 1: a) Depiction of the hanged cadaver; b) visual representation of the spatial treatments related to the collection of entomological evidence.

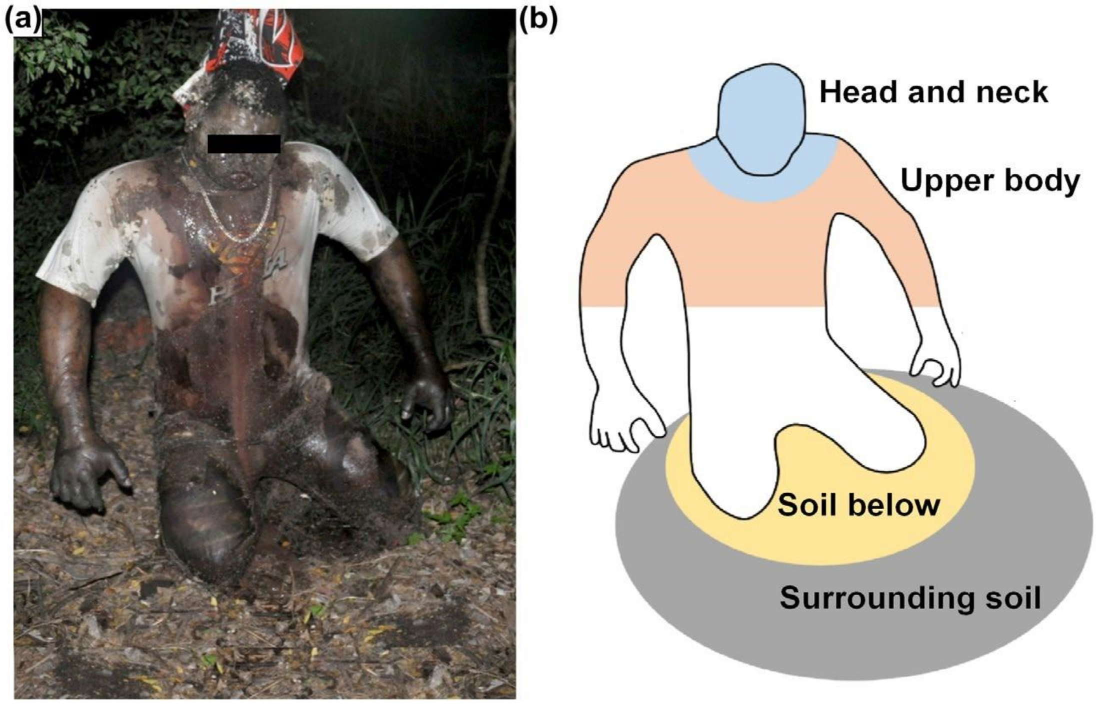

Figure 2 – Daily cumulative (%) emergence of the three most abundant species sampled, related to time (days) after collection in the field